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RESEARCH ARTICLE

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α-/β-Glucosidase and α-Amylase Inhibitory Activities of Roselle (*Hibiscus sabdariffa* L.) Ethanol Extract

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Background: Diabetes mellitus is a metabolic disease, characterized by hyperglycemia due to disturbance in both insulin secretion and function. One of theurapeutic approaches is to reduce blood glucose levels by inhbiting α-/β-glucosidase and α-amylase involved in carbohydrate digestion. Thus, inhibition of these enzymes play important role in the treatment of diabetes mellitus. Roselle (*Hibiscus sabdariffa* L.) has been known to have several medicinal properties and potency as an antidiabetics agents. This reseacrh aimed to observe antidiabetic properties of roselle ethanol extract (REE) towards α-glucosidase, β-glucosidase and α-amylase.
Materials and Methods: REE was done with maceration technique using diluent of 70% ethanol. Antidiabetic properties were measured by inhibitory activity of α-amylase, α-glucosidase and β-glucosidase.
Results: REE was able to inhibit α-/β-glucosidase and α-amylase in the highest concentration with inhibition percentage of 72.68, 47.34 and 73.08% respectively, and were comparable with Acarbose of 81.49, 50.97, 73.08%. The median inhibitory concentration (IC_{50}) of α-/β-glucosidase and α-amylase of REE were 15.81, 41.77, 18.09 µg/mL respectively, and Acarbose were 9.45, 22.57, 3.64 µg/mL respectively.
Conclusion: REE inhibits α-/β-glucosidase and α-amylase.

Keywords: Roselle, Acarbose, α-glucosidase, β-glucosidase, α-amylase, antidiabetic

Introduction

Diabetes mellitus (DM) is a metabolic disease, characterized by hyperglycemia due to disturbance in both insulin secretion and function.[1] Type 1 DM is a type with defective insulin secretion by pancreatic β-cells, and type 2 DM is a type with insulin resistance (a condition in which peripheral cells do not respond normally to insulin) or β-cell dysfunction.[2] DM has postprandial phase indicated by increased blood glucose levels, which is called postprandial hyperglycemia.[3]

Antihiperglycemia is commonly used to reduce hyperglycemia prostaglandins via inhibition of responsible enzymes in glucose absorption that hydrolize carbohydrates, such as α-/β-glucosidase and α-amylase.[4] Glucosidase is an enzyme involved in complex carbohydrate dissociation in the small intestine that play its role to cleave glicosidic bonds, resulting in glucose release from the non-reducing end of an oligo- or poly-saccharide chain involved in glycoprotein biosynthesis.[4]

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Common drugs are expensive or generate adverse effects. Common adverse effects of enzyme-inhibitory drugs, like Acarbose, are excessive inhibition of pancreatic α-amylase, which can result in abdominal distention, flatulence and diarrhea.[5] Thus, natural medicine is proposed to be an alternative. Traditional herbal medicine is believed to possess hypoglycemic activity. There are more than 800 species of plants reported to contain hypoglycemic properties.[6]

Roselle (*Hibiscus sabdariffa* L) has been used as a medicinal beverage in many countries such as Australia, India, Myanmar, Thailand, Senegal, France, Gambia, Nigeria, Greece, Saudi Arabia, Sudan, Latin America, Panama, Indonesia, Malaysia and China.[7] Bioactive compounds is documented in roselle such as alkaloids, anthocyanins, flavonoids, saponins, steroids, sterols and tannins.[8] Roselle extract demonstrate anti-insulin resistance properties due to its activity in reducing hyperglycemia and hyperinsulinemia, as well as decrease in Low Densisty Lipoprotein (LDL) and High Density Lipoprotein (HDL).[9] Roselle extract also play role in lowering blood glucose levels in streptozotocin-induced diabetic mice, it is comparable with glibenclamide.[6,10,11] Therefore our current study was carried out to evaluate possible effectivity of Roselle extract to inhibit α/β-glucosidases and α-amylase.

Materials and methods

Preparation roselle extract

Extraction was performed based on maceration method.[12,13] Roselle calyx were collected from Cimenyan, West Java, Indonesia. The plant was identified by herbarium staff of Department of Biology, School of Life Sciences and Technology, Bandung Institute of Technology, Indonesia. Dried and milled roselle calyx were soaked in 70% distillated ethanol and filtered every 24 hours. The process was performed until colorless filtrate was gained, then filtrate was evaporated to obtain ethanol extract. Roselle ethanol extract (REE) was stored at -20°C.

α-glucosidase inhibitory activity assay

The α-glucosidase inhibitor activity was tested with the modified method.[1,14,15] Mixture reaction consists of 4-Nitrophenyl α-D-glucopyranoside (N1377, Sigma-Aldrich, St.Louis, USA), phospate buffer (pH 7.0) and sample solution. After mixed well, α-glucosidase was added, incubated at 37°C for 30 minutes, then Na_2CO_3 was added. The 4-Nitrophenyl α-D-glucopyranoside was measured at 400 nm wavelength with microplate reader The inhibition percentage of α-glucosidase was calculated according to equation 1:

$$\% \text{ inhibition} = \frac{(C-S) \times 100}{C}$$

C : absorbance of control
S : absorbance of sample

β-glucosidase inhibitory activity assay

The β-glucosidase inhibitory activity assay was conducted using modified method.[1] Mixture solution contained 4-Nitrophenyl β-D-glucopyranoside (N7006, Sigma-Aldrich), phosphate buffer (pH 7.0) and sample solution. Mixture reaction was incubated at 37°C for 5 minutes, added with enzyme mixture and incubated for 15 minutes. Reaction was stopped by addition of Na_2CO_3. Absorbance of 4-Nitrophenyl β-D-glucopyranoside was measured at 400 nm wavelength. The inhibition percentage of β-glucosidase was calculated according to equation 1.

α-amylase inhibitory activity assay

The α-amylase inhibitory activity assay was performed using a modified method.[16] Sample was introduced into sample well, dimethyl sulfoxide (DMSO) was used as a blank. Furthermore, α-amylase enzyme (A7595, Sigma-Aldrich) was added into each well, except for blank well. Then the mixture was incubated at 37°C for 10 minutes, and added with starch solution in each well, while control well was added with phospate buffer. Another incubation at 37°C for 15 minutes was conducted. Enzymatic reaction was stopped by adding acidic iodine solution in each well. The absorbance was measured at 565 nm wavelength. The inhibition percentage of α-amylase was calculated according to equation 1.

Statistical analysis

Data was presented as mean±standard deviation. To compare treatments, analysis of variance (ANOVA) was used, and $p<0.05$ were considered as statistically significant, along with Duncan Post-Hoc Test significant and 95% confidence interval. The median inhibitory concentration (IC_{50}) was measured to determine the inhibitory activies of α/β-glucosidase, α-amylase, according to linear regression. SPSS version 20.0 program was used for statistical analysis.

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Results

α-glucosidase inhibitory activity

The α-glucosidase inhibitory of REE and Acarbose can be seen in Table 1 and Figure 1, and while the value of IC_{50} can be seen in Table 2.

α-glucosidase activity of REE and Acarbose occurred in concentration-dependent manner in which higher α-glucosidase inhibition present in higher concentration of sample. Figure 1 shows that at the highest concentration (37.5 µg/mL), Acarbose generated higher inhibitory activity, compared with REE. The IC_{50} value of Acarbose 14.65 µM or 9.45 µg/mL was more active than REE 15.81 µg/mL (Table 2). Inhibition of α-glucosidase is expected to normalize blood glucose level.[17] Based on statistical analysis, both REE and Acarbose had significant α-glucosidase inhibition activity differences at each concentration, higher concentration increased the activity (p<0.05).

β-glucosidase inhibitory activity

The β-glucosidase is a carbohydrate hydrolizing enzyme associated with metabolic disorder such as DM. Inhibition of carbohydrate hydrolyzing enzymes is used as therapeutic approach to decrease hyperglucose level and improve hyperglicemic.[1,18] The β-glucosidase inhibitory of REE and Acarbose can be seen in Table 3 and Figure 2, the IC_{50} value can be seen in Table 4. The result of β-glucosidase activity in REE and Acarbose showed that inhibitory activity was associated with concentrations in which higher β-glucosidase inhibition present in higher concentration of sample. Inhibition percentage of Acarbose with percentage 50.97±3.78 was comparable with REE 47.34±2.69 (Table 3). As shown in Table 4, Acarbose was more active in inhibiting β-glucosidase, the IC_{50} value 34.96 µM or 22.57 µg/mL was lower compared to REE 41.77 µg/mL. Statistical analysis showed REE and Acarbose had significant differences in β-glucosidases inhibition activity at each concentration, higher concentration increased β-glucosidase inhibition (p<0.05).

α-amylase inhibitory activity

Pancreatic α-amylase is an enzyme in the digestive system and catalyses the initial step in starch hydrolysis.[19] Inhibition of intestinal α-amylase delays the starch and oligosaccharides degradation to monosaccharides prior to absorbtion, which later decreases the glucose absorption, as well as postprandial blood glucose level.[20,21] The α-amylase activity showed inhibitory activity in concentration-dependent manner in which increasing concentrations generated higher inhibitory activity. Inhibitory activity was lower in REE compared to Acarbose (Table 5 & 6, Figure 3) with inhibition percentage 73.08±3.85 of REE while Acarbose 77.08±4.77. Acarbose was more active in inhibiting α-amylase, the IC_{50} value 5.64 µM or 3.64 µg/mL was more active compared to REE 18.09 µg/mL. Based on statistical analysis, both REE and Acarbose had significant difference in α-amylases inhibition activity at each concentration, higher concentration increased α-amylase inhibition (p<0.05).

Table 1. Effect concentrations toward α-glucosidase inhibitory activity (%) of REE and Acarbose

Concentrations (µg/mL or µM)	Inhibitory Activity (%)	
	REE	Acarbose
37.5	72.68±1.56 [e]	81.49±1.19 [e]
12.5	46.38±2.72 [d]	44.94±0.54 [d]
6.25	41.63±0.74 [c]	38.21±0.32 [c]
3.125	38.79±0.26 [b]	34.86±0.43 [a]
1.563	33.16±1.00 [a]	32.82±0.83 [a]
0.781	32.48±1.05 [a]	32.44±2.82 [a]

The data are presented as mean±standard deviation. Different superscript small letters ([a,b,c,d,e]) indicate significance in the same column among concentrations of samples, used Duncan's post hoc test at p<0.05. Each treatment of samples was performed in triplicate.

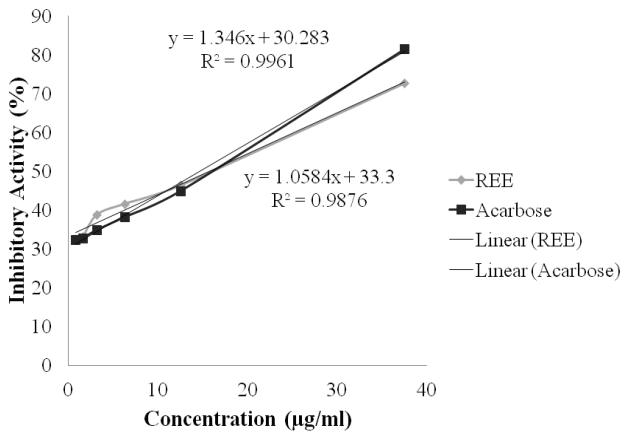

Figure 1. The α-glucosidase inhibitory activity (%) of REE and Acarbose. REE and Acarbose were diluted in 10% DMSO to reach the final concentrations of 37.5, 12.5, 6.25, 3.125, 1.563, 0.398, 0.781 µg/mL.

Table 2. The median inhibitory activity (IC_{50}) of α-glucosidase of REE and Acarbose

Sample	Equation	R^2	IC_{50} (µg/mL/µM)	Average IC_{50} (µg/mL/µM)
REE Test 1	Y = 1.0166x + 33.096	0.9866	16.63 µg/mL	
REE Test 2	Y = 1.0296x + 33.828	0.9845	15.71 µg/mL	
REE Test 3	Y = 1.1288x + 32.976	0.9802	15.08 µg/mL	
Average	y = 1.0584x + 33.3	0.9876	15.77 µg/mL	15.81 ± 0.78 µg/mL
Acarbose Test 1	Y = 1.3975x + 29.574	0.9977	14.62 µM (9.43 µg/mL)	
Acarbose Test 2	Y = 1.3267x + 31.097	0.9870	14.25 µM (9.43 µg/mL)	
Acarbose Test 3	Y = 1.3139x + 30.179	0.9955	15.09 µM (9.74 µg/mL)	
Average	y = 1.346X + 30.283	0.9961	14.64 µM (9.45 µg/mL)	14.65 ± 0.42 µM (9,45 µg/mL)

The IC_{50} value was presented as mean ± standard deviation in each replication of samples (test 1, 2, 3) and average from 3 replication.

Table 3. Effect concentrations toward β-glucosidase inhibitory activity (%) of REE and Acarbose

Concentrations (µg/mL or µM)	Inhibitory Activity	
	REE	Acarbose
37.5	47.34±2.69 ^{d}	50.97±3.78 ^{e}
12.5	23.47±1.49 ^{c}	32.54±3.17 ^{d}
6.25	21.66±0.62 ^{bc}	23.81±0.23 ^{c}
3.125	19.56±0.99 ^{ab}	22.35±1.06 ^{c}
1.563	16.92±3.32 ^{a}	15.60±1.00 ^{b}
0.781	16.39±0.85 ^{a}	10.60±1.93 ^{a}

The data are presented as mean±standard deviation. Different superscript small letters (^{a,ab,b,bc,c,d,e}) indicate significance in the same column among concentrations of samples, used Duncan's post hoc test at $p<0.05$. Each treatment of samples was performed in triplicate.

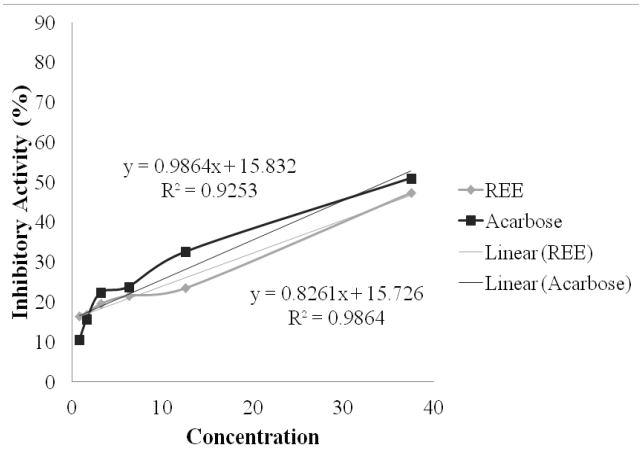

Figure 2. The β-glucosidase inhibitory activity (%) of REE and Acarbose. REE and Acarbose were diluted in 10% DMSO to reach the final concentrations of 37.5, 12.5, 6.25, 3.125, 1.563, 0.398, 0.781 µg/mL.

Discussion

The result of present study showed that REE possessed antidiabetic activity, based on inhibitory activity of α-/β-glucosidase and α-amilase. These results indicate the antidiabetic properties of REE was lower than Acarbose. The α-/β-glucosidase are carbohydrate hydrolyzing enzymes that take place in metabolic disorder such as DM. Glucosidase cleaves the glycosidic bond that release glucose from the non-reducing end of an oligo- or poly-saccharide chain involved in glycoprotein biosynthesis.[22] Inhibition of carbohydrate hydrolyzing enzymes is therefore used as therapeutic approach to decrease hyperglycemia.[23] This data is in accordance with previous study that the aqueous extract of roselle has the inhibitory activity of α-glucosidase as well as lowering blood glucose levels in DM animal models.[24]

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Table 4. The median inhibitory activity (IC_{50}) of β-glucosidase of REE and Acarbose

Sample	Equation	R^2	IC_{50} (µg/mL/µM)	Average IC_{50} (µg/mL/µM)
REE Test 1	Y = 0.9265x + 14.298	0.9722	38.53 µg/mL	
REE Test 2	Y = 0.8285x + 15.843	0.9652	41.23 µg/mL	
REE Test 3	Y = 0.7235x + 17.036	0.9944	45.56 µg/mL	
Average	y = 0.8264x + 15.726	0.9864	41.48 µg/mL	41.77 ± 3.55 µg/mL
Acarbose Test 1	Y = 1.0059x + 16.285	0.9240	33.52 µM (21.64 µg/mL)	
Acarbose Test 2	Y = 1.0905x + 15.473	0.9075	31.66 µM (20.43 µg/mL)	
Acarbose Test 3	Y = 0.8627x + 15.738	0.9381	39.71 µM (25.63 µg/mL)	
Average	y = 0.9864x + 15.832	0.9253	34.63 µM (22.35 µg/mL)	34.96 ± 4.22 µM (22.57 µg/mL)

The IC_{50} value was presented as mean ± standard deviation in each replication of samples (test 1, 2, 3) and average from 3 replication.

Table 5. Effect concentrations toward α-amylase inhibitory activity (%) of REE and Acarbose

Concentrations (µg/mL or µM)	Inhibitory Activity	
	REE	Acarbose
34.29	73.08±3.85 [c]	77.08±4.77 [d]
11.43	41.88±6.45 [b]	58.82±5.88 [c]
5.71	38.00±4.00 [b]	55.83±3.82 [c]
2.86	25.49±3.92 [a]	52.67±4.16 [c]
1.43	22.42±2.78 [a]	43.86±1.75 [b]
0.71	20.24±2.73 [a]	35.45±0.92 [a]

The data are presented as mean±standard deviation. Different superscript small letters ([a,b,c,d]) indicate significance in the same column among concentrations of samples, used Duncan's post hoc test at $p<0.05$. Each treatment of samples was performed in triplicate.

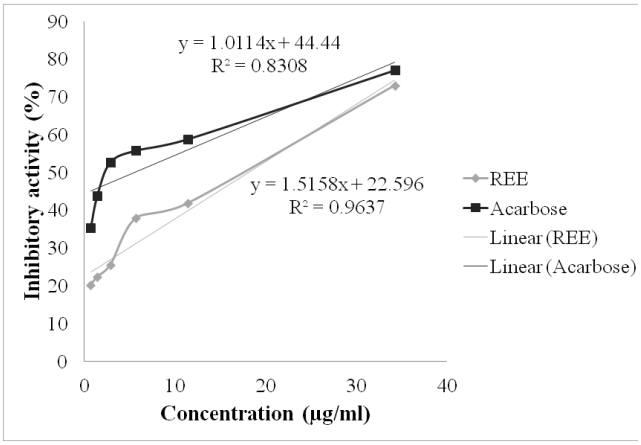

Figure 3. The α-amylase inhibitory activity (%) of REE and Acarbose. REE and Acarbose were diluted in 10% DMSO to reach the final concentrations of 37.5, 12.5, 6.25, 3.125, 1.563, 0.398, 0.781 µg/mL.

Roselle ethanol extract reduced the blood glucose levels on diabetic mice was comparable with n-hexane and ethyl acetate extract of roselle.[6] The ability might be due to presence of dietary phytochemicals in roselle such as anthocyanins, flavonoids and phenolic acids.[25] Roselle also has variety content bioflavonoid such as prunin, myrciacitrin, 6-hydroxyl-flavonoids, 6-hydroxyluteolin, apigenin, and luteolin, that can lower blood glucose, and have inhibitory activity of α-glucosidase.[24] Flavonoid contents of REE are flavonols (gossypetin) and the anthocyanins.[25] Flavonoid and isoflavonoid glycosides are found in human small intestine, in which α- and β-glucosidase inhibitory present to inhibit carbohydrate catalyses[26] that suggests contribution of flavonoid content as antidiabetic effects.

In this study, REE has α-amylase inhibitory activity. Inhibition of α-amylase will promote retardation of starch hydrolysis, that reduces digestion and absorption rate

Table 6. The median inhibitory activity (IC_{50}) of α-amylase of REE and Acarbose

Sample	Equation	R^2	IC_{50} (μg/mL/μM)	Average IC_{50} (μg/mL/μM)
REE Test 1	Y = 1.5748x + 22.019	0.9858	17.77 μg/mL	
REE Test 2	Y = 1.4414x + 23.607	0.8631	18.31 μg/mL	
REE Test 3	Y = 1.5512x + 22.161	0.9454	18.18 μg/mL	
Average	Y = 1.5158x + 22.596	0.9637	18.07 μg/mL	18.09 ± 0.28 μg/mL
Acarbose Test 1	Y = 0.8853x + 42.773	0.8291	8.16 μM (5.26 μg/mL)	
Acarbose Test 2	Y = 1.0349x + 46.243	0.7196	3.63 μM 2.10 μg/mL	
Acarbose Test 3	Y = 1.1141x + 44.305	0.8909	5.11 μM (3.29 μg/mL)	
Average	Y = 1.0114x + 44.44	0.8308	5.49 μM (3.54 μg/mL)	5.64 ± 2.31 μM (3.64 μg/mL)

The IC_{50} value was presented as mean ± standard deviation in each replication of samples (test 1, 2, 3) and average from 3 replication.

of carbohydrates, resulting in decreased post-prandial hyperglycemia.[15] Flavanoids content in roselle play key role in α-amylase inhibitory activity.[27] Inhibition of α-amylase preserve β-cell integrity and function by removing free radicals could enhance protection against the progression of insulin resistance in type 2 DM.[28] Previous studies also showed that roselle extract has potency to inhibit α-amylase activity, sugars and starch absorption, which may assist in weight loss.[29]

Flavonoids present in roselle, are well known to contain medicinal properties such as antiinflammatory, antioxidant and antidiabetic.[30,31] Phenolic and flavonoids content in roselle are suggested to play role in inhibitory activity of both α-amylase and α-/β-glucosidase.[32,33] Interactions between chemical compound of REE and enzymes causes alteration in the enzyme's molecular configuration, as well as hydrophilic and hydrophobic properties, that will decrease enzyme activities.[10] Active components of REE compete with the substrate in binding to active site of enzyme prevent the oligosaccharides break-down to disaccharides.[34]

Conclusion

REE is a promising antidiabetic agent to inhibit α-/β-glucosidase and α-amylase. Further researches regarding identification and development of bioactive compounds contained in roselle, are encouraged.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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